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CALCULATING ANCESTRAL INFLUENCE IN MAN

A MATHEMATICAL MEASURE OF THE DEMONSTRATED FACTS OF BI-SEXUAL HEREDITY

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By ancestral influence is meant the contribution of definite hereditary, mental, physical and moral qualities, by a given ancestor to a given descendant. Pedigree-studies have set forth in man, as in many other organisms, rules of inheritance of many specific traits or qualities. Cytological investigations have shown how chromosomes duplicate, segregate and recombine (sometimes crossing-over and showing other special phenomena) in the course of gametogenesis and fertilization.

In bi-sexual species of plants or animals, the germ-plasm is not indefinitely dilutable or divisible as many genealogists have assumed, but its dilutability and divisibility are limited by the number of chromosome-characteristics of the gametes of the particular species. In the human zygote two gametes each with 12 chromosomes unite. This means that, barring crossing-over, mutation and other special phenomena, normally the human germ-plasm, and consequently all hereditary human qualities, that is, the "blood," is divisible only into 24 unequal units corresponding to the 24 zygotic chromosomes.

Note—There is still disagreement among cytologists concerning the number of chromosomes in man.

Wieman, H. L., "The Chromosomes of Human Spermatocytes," *Amer. J. Anat.*, **21**, No. 1, January, 1917. This paper concludes that in man the zygotic or diploid number of chromosomes is 24, of which 2 are the *xy* idiochromosomes.

von Winiwarter, H., "Etudes sur la spermatogenèse humaine," *Arch. Biol.*, **27**, 1912. This paper concludes that the zygotic number of chromosomes in the human male is 47, in the human female, 48.

See also Stevens, Guyer, Montgomery and Evans.

When this problem is settled beyond peradventure, the correct number can be used in the accompanying formulas which are here stated in general terms.

It is well demonstrated that the chromosomes are composed of genes or the determiners of natural inheritance. Each chromosome is a gene-

radical or linkage-group of trait determiners. The chromosomes are the germ-plasm. If then it is sought to measure the influence of a given ancestor upon the hereditary traits of a given propositus, it is necessary to develop a series of mathematical formulas which measure the probabilities of definite situations happening in reference to the transmission of chromosomes from the given ancestor to the given propositus.

In developing such formulas the following factors must be taken into consideration:

First. Whether the species in question is bi-sexual.

Second. Whether in the particular species the male or the female is the heterozygous sex.

Third. Due provision must be made for sex-linked traits.

Fourth. The number of chromosomes, both gametic and zygotic, characteristic of the species, must be known.

Fifth. For the present the individual chromosomes will not be weighed, but simply counted. Doubtless the ontogenetic working out of each chromosome is very specific both qualitatively and quantitatively. When ultimately genetical and cytological research locate in definite chromosomes the genes for definite traits, then the several chromosomes can be weighted, and due provision for their differential values properly made in the formulas for measuring the several features of ancestral influence.

Sixth. Due cognizance must be taken of the possibility of mutation, crossing-over, non-disjunction and other special phenomena occurring in the course of the descent of chromosomes from a given ancestor to the propositus. In the accompanying formulas such phenomena are not provided for, because their rates of occurrence are not yet measurable in man, but when one by one their mathematical measures are attained, each such measure can be woven into the accompanying foundation formulas. The present formulas will not be upset by such corrections, but simply modified in the direction of greater accuracy.

Seventh. It must be considered that in a given gamete a definite chromosome may have been derived from either the paternal or the maternal source. This is especially important in cases of zygotes of mixed blood.

Eighth. In certain situations it will be necessary to specify whether a given trait is dominant or recessive to its allelomorph.

Finally, all formulas must be stated in general terms, so that they may apply with equal accuracy to any species concerning which chromosomal data are at hand.

There are many features of ancestral influence which may be mathematically formulated. Among them are (1) the average contribution in number of chromosomes that a given ancestor, taken as a type, will contribute to the propositus; (2) the range in such numbers; (3) the probability that the given ancestor, taken as a type, will contribute all of the hereditary influences which the particular half-pedigree furnishes to the

propositus; (4) the probability that the particular propositus, and consequently all of his or her direct ancestors, will be entirely eliminated as a contributor to the hereditary constitution of the propositus; (5) that a definite number of chromosomes specified as to maternity or paternity of origin, carried by a given ancestor, will by such ancestor be passed on to the propositus; (6) as one of many special cases, the probability that a given ancestor who appears once or more in each the maternal and paternal half-pedigree, will be duplicated exactly in zygotic chromosomal constitution by the propositus.

The principles set forth in the paper of which this is an abstract make possible the mathematical formulation of an indefinitely great number of complex situations in reference to ancestral influence. The completer paper presents in detail twenty-seven such formulas. Eight of these (numbers 1, 4, 5, 6, 7, 8, 9 and 10) for each individual pedigree-place, to which the particular formulas apply, were worked out for each member of the first five ancestral generations in man and shown on charts, entitled "Ancestral Influence in the Human Male," and "Ancestral Influence in the Human Female." (In the accompanying charts bearing the above titles these same formulas are worked out for the first three ancestral generations only.) These particular calculations in reference to man are based upon the following data and conditions: (*a*) that the gametic number of human chromosomes is 12; the zygotic or somatic number in each sex 24; (*b*) that the male is heterozygous (*xy*) and the female homozygous (*xx*) in reference to sex; (*c*) that all of the purely hereditary qualities are carried by the several chromosomes; and (*d*) that in the first or foundation formulas no provision will be made for mutation, crossing-over, non-disjunction or other special phenomena.

These eight formulas worked out in detail on the human pedigree-charts follow:

No. 1. A = Average number of chromosomes passed on by the selected ancestor, taken as a type, to the F_1 zygote, i. e., the propositus.

A = Also the average number of chromosome-pairs each of which contributes one member to the diploid or somatic group of chromosomes of the F_1 zygote.

In man the diploid or somatic number of chromosomes is taken tentatively at 24, i. e., 11 equivalent pairs and one *xx* or *xy* sex-pair.

$$A = \frac{n}{2^{(p-1)}} + \left\{ \begin{array}{l} (C_y \text{ or } C_x \text{ in case of ancestors from whom an } x\text{- or a} \\ \qquad y\text{- chromosome } \textit{may} \text{ descend.}) \end{array} \right.$$

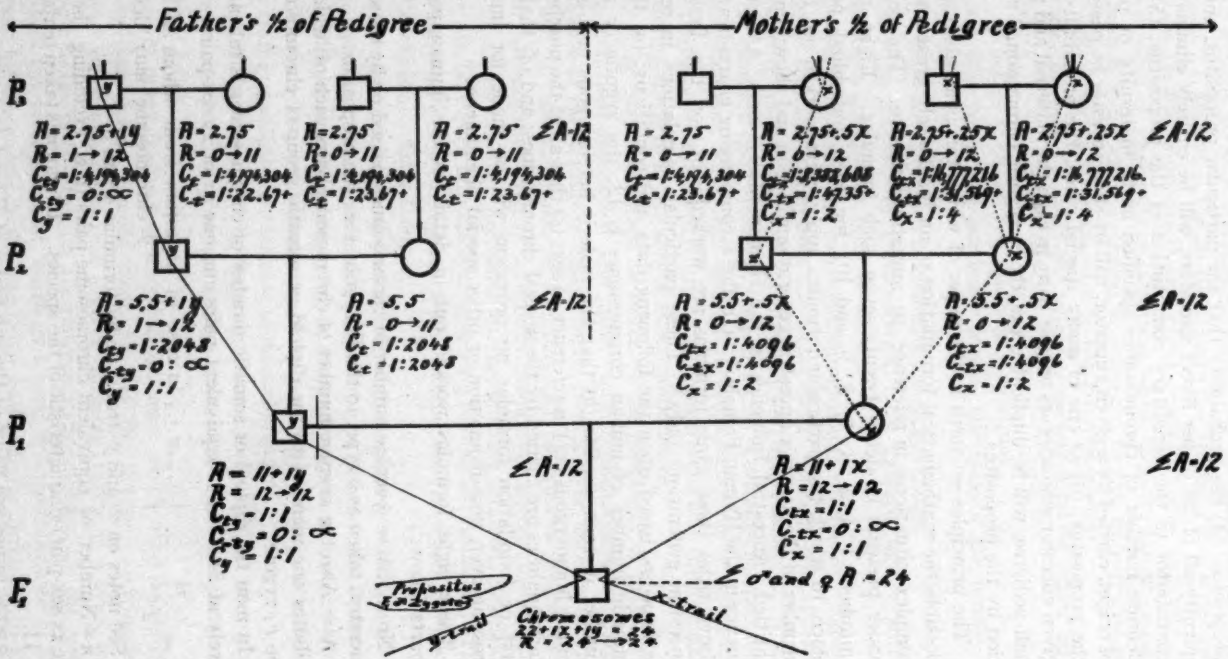
See notes on *x*- and *y*- trails, under formulas 4 and 5.

n = Number of equivalent chromosome-pairs (i. e., excluding the *xy* or the *xx* sex-pair) characteristic of the species. In man n is taken tentatively at 11.

p = the numerical value of the sub-figure of P used in pedigree-manner

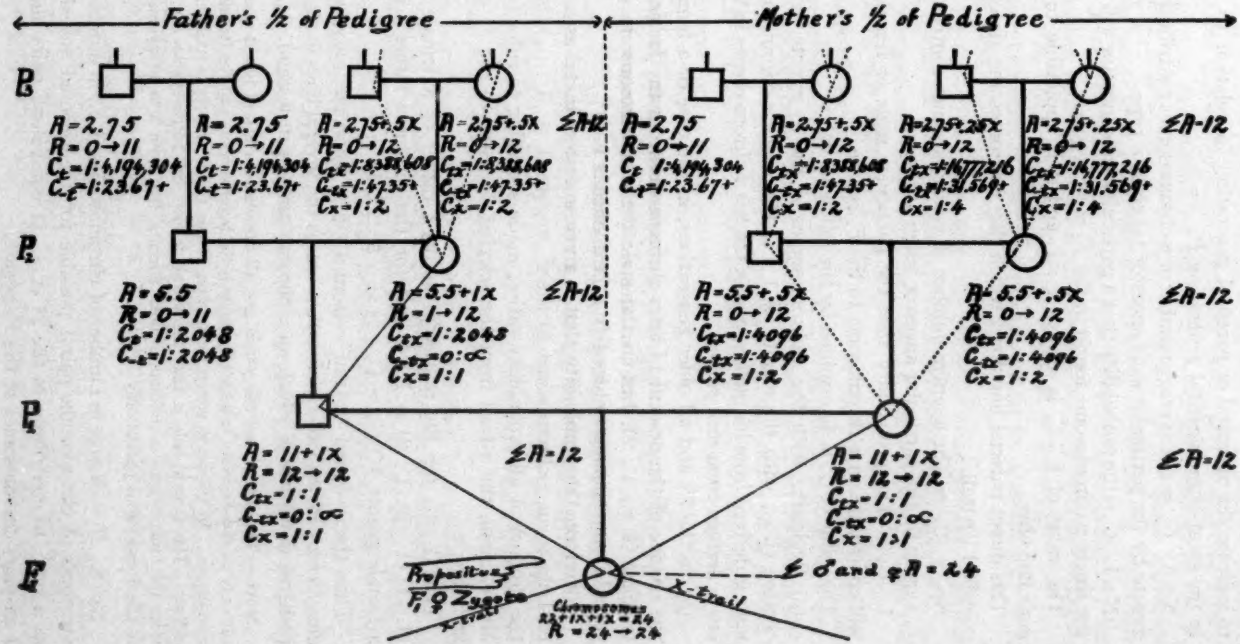
1. Ancestral Influence in the Human Male

based upon average, range and chance in the contribution of chromosomes to the F_1 Zygote.



2. Ancestral Influence in the Human Female

based upon the average, range and chance of contribution of chromosomes to the F_1 Zygote.



to indicate the ancestral or parental generation in relation to F_1. Thus in the great-grandparental generation P_3, $p=3$.

No. 4. C_y = the average number of y-chromosomes contributed to the F_1 zygote by the particular *male* ancestor taken as a type.

No. 1. C_y = the probability that a particular *male* ancestor will contribute the single y-chromosome found in the F_1 *male* zygote.

The value of $1 : C_y$ is always 1 : 1, and it is applicable to the *direct male* line only.

This direct paterial line down which the y-chromosome descends is the so-called "y-trail."

No. 5. C_x = The average number of x-chromosomes contributed to the F_1 zygote by the particular ancestor taken as a type.

$1 : C_x$ = The probability that the particular male or female ancestor will contribute an x-chromosome to the F_1 zygote.

The value of C_x is determined by the following rule:

(*a*) Beginning with the x-chromosomes in the F_1 zygote, without exception (i. e., with the chance of 1 : 1) the *single* x-chromosome in the *male* is derived from his *mother;* and of the *two* x-chromosomes in the *female*, *one* is derived from *each parent.*

(*b*) In the P_1 and all older generations, in the case of a female zygote the chance of the descent of an x-chromosome from the *father* or *mother* is *equal* (i. e., 1 : 2), but in the *male* the x-chromosome is always received from the *mother* alone (i. e., the chance is 1 : 1).

Therefore, the probability that a given ancestor, taken as a type, will contribute an x-chromosome to the F_1 zygote (i. e., $1 : C_x$), ise qual to the product of all probability ratios, made according to rules (*a*) and (*b*) above, along the x-trail from the selected ancestor to the P_1 generation inclusive.

Thus $1 : C_x$ for the mother's mother's father of a female zygote = $(1 : 1).(1 : 2).(1 : 2) = 1 : 4$; but for the mother's father's mother of a female zygote $1 : C_x = (1 : 1).(1 : 2).(1 : 1) = 1 : 2$.

Thus the so-called "x-trail"—from a *son to the mother only*, but from a *daughter to both the father and mother*, traces the several lines in the ancestral pedigree down *one* of which an x-chromosome *must* descend.

NOTE.—The x-trail and the y-trail and all calculations herein based upon them apply only to those species in which the male is the heterozygous and the female the homozygous sex. In species in which the reverse is true the x- and the y-trails exchange positions. The general rule is that the y-trail follows the heterozygous sex; the x-trail from the homozygous individual to both parents, but from a heterozygous individual to the homozygous parent only.

No. 6. R = Range in number of chromosomes (i. e., in number of single members of chromosome-pairs) possible for the selected ancestor, taken as a type, to contribute to the 24 (i. e., 11 equivalent pairs and 1 xy or 1 xx pair) chromosomes of the F_1 zygote.

In a parent of the P_1 generation, $R = 12 \longrightarrow 12$, i. e., there is no range in the number of chromosomes which the given parent may contribute to the F_1 zygote; this number is always 12. For all ancestors of the P_2 and all older generations, $R = 0 \longrightarrow 11$, except (*a*) for those ancestors from whom an *x*- or a *y*-chromosome *must* certainly descend (solid line *x*-trail or *y*-trail in chart), in whom $R = 1 \longrightarrow 12$; except also (*b*) for those ancestors from whom an *x*-chromosome *may* by alternate chance, descend (dotted line *x*-trail in chart) in whom $R = 0 \longrightarrow 12$.

In a general formula 11 would be substituted for by n, and 12 by $n + 1$, with value as in formula 1.

No. 7. $1 : C_l$ = The probability that *one or the other* member of *each* of *all* of the *equivalent* chromosome-pairs characteristic of the species (i. e., an entire chromosome-series excluding the *xx* and *xy* pairs), from the given ancestor will enter the F_1 zygote.

In the case of ancestors not in the *x*-trail or the *y*-trail, such an occurrence is the extreme limit of ancestral influence possible to a given ancestor, and its happening precludes any hereditary influence at all on the propositus, i. e., the F_1 zygote, on the part of any other ancestor of the same paternal or maternal half-pedigree, as the case may be, except the lineal ancestors and descendants of the given ancestor.

$1 : C_l = 1 : (2^n)^{p-1}$

n = Value as in formula 1. In man n is taken tentatively at 11.

p = Value as in formula 1.

No. 8. $1 : C_{lx}$ or $1 : C_{ly}$ = The probability that *one or the other* member of *each* of the several equivalent chromosome-pairs characteristic of the species, and *also* one *x*- or one *y*-chromosome, as the case may be, from the given ancestor will enter the F_1 zygote. (In man this total gametic number of chromosomes is taken tentatively at 12.)

Such an occurrence can happen only in reference to ancestors in the *x*- or *y*-trails, and its happening precludes any hereditary influence at all on the propositus, i. e., the F_1 zygote, on the part of any other ancestor of the same paternal or maternal half-pedigree, except the lineal ancestors and descendants of the given ancestor.

$1 : C_{lx} = (1 : C_l).(1 : C_x)$

Values of $1 : C_l$ and $1 : C_x$ as in formulas 5 and 7.

$1 : C_{ly} = (1 : C_l).(1 : C_y)$

Values of $1 : C_l$ and $1 : C_y$ as in formulas 4 and 7.

No. 9. $1 : C_{-l}$ = The probability that *no member* of *any* of the equivalent chromosome-pairs characteristic of the species (thus barring for the time being all consideration of the *xx* or *xy* chromosome-pairs) from the given ancestor will enter the F_1 zygote.

In such an occurrence the given ancestor and all of his or her lineal ancestors are *entirely eliminated* as contributors to the natural inheritance of the propositus, i. e., the F_1 zygote, *provided* that such given

ancestor is not in the x-trail or y-trail, and does not, due to inbreeding, appear elsewhere in the ancestral pedigree.

$$1 : C_{-t} = 1 : \left(\frac{2^{p-1}}{2^{p-1}-1}\right)^{n}$$

n = Value as in formula 1.

p = Value as in formula 1.

No. 10. $1 : C_{-tx}$ = or $1 : C_{-ty}$ = The probability that no member of any equivalent chromosome-pair, *nor* of the xx or xy chromosome-pair, from the given ancestor will enter the F_1 zygote, i. e., the probability (barring crossing-over and other special phenomena, and the appearance of the given ancestor elsewhere in the pedigree), of the *complete elimination* of the hereditary influence of the given ancestor and all of his or her lineal ancestors, on the propositus, i. e., the F_1 zygote.

This formula is applicable *only* to those ancestors *in the x-trail or y-trail.*

$1 : C_{-tx} = (1 : C_t).(1 : C_{-x})$

$1 : C_{t-y} = (1 : C_{-t}).(1 : C_{-y})$

Values of $1 : C_{-t}$ as in formula 9

$$C_{-x} = \frac{C_x}{C_{x-1}}$$

$$C_{-y} = \frac{C_y}{C_{y-1}}$$

Summary.—The present problem of measuring ancestral influence by tracing chromosomes or gene-s-radical is demonstrated to be a special case under the following general situation:

Given: First. A single unit factor (a gene) in a known situation (in the P_1 generation) and the quotient measuring the probability of its reacting in a definite manner or reaching a definite new situation at the end of a single system of moves or transformations (in the F_1 generation).

Second. The demonstrated synchronization or sequence in the systems of moves or transformations of two or more such unit factors.

By applying the correct principles of combination and chance, the probability that a given complex situation will result from a given set of constituent conditions at the end of a given number of definite kinds of transformations may be mathematically formulated.

In case of measuring ancestral influence by tracing chromosomal descent, the situations and transformations concern definite chromosome-complexes and their breaking-up, their re-combination and their transmission to offspring.

The formulas here set forth are foundational. They are stated in general terms whose validity depends upon their presenting correct mathematical pictures of the chromosomal processes which work out in the germ-cell cycle.

THE EFFECT OF ANESTHETICS ON LIVING CELLS

BY GRACE MEDES AND J. F. MCCLENDON

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Communicated by A. G. Mayor, March 17, 1920

The object of this research is to determine the effect of different anesthetics on several activities or properties of living cells, permeability, oxygen consumption, CO_2 production, photosynthesis, protoplasmic streaming and cell structure. Plant cells, Elodea as well as animal cells, Cassiopea, were used. Not all anesthetics had the same effect and the same anesthetic affected the same activity of a plant differently from an animal and affected different activities of the same cell differently. One correlation, however, was evident. All the anesthetics tried increased plant cell respiration and permeability. What might seem to be exceptions to this rule occurred under overdoses of the anesthetic and we were dealing with dead cells, which are outside the limits of this research.

All of the experiments were performed in thermostats at 30°. The Cassiopeas were kept in sea water, usually of alkaline reserve = 0.0025 N and p_H = 8.2 at the start, and the Elodea in a distilled water solution of $NaHCO_3$ of alkaline reserve = 0.0025 N and p_H = 7.6. At this alkaline reserve a change in p_H of 0.1 was produced by a change in CO_2 content per liter of 1.5 cc. By determining the change in p_H colorimetrically the production of CO_2 could be estimated. The oxygen consumed was determined by the Winkler method. The results were expressed in cc. of tenth normal thiosulphate for 100 cc. water in the Elodea experiments and as cc. of O_2 in the Cassiopea experiments. Since these data cover many pages even when condensed into tabular form, it is thought best to convert the results into percentages of the normal and summarize them. In the first place, higher concentrations of anesthetics evidently killed the cells and it was necessary to decide at just what concentration permanent injury occurred in order to separate such data from that concerning reversible changes characteristic of anesthesia. In the higher concentrations of anesthetics Elodea cells became irreversibly plasmolyzed (false plasmolysis), respiration and photosynthesis ceased, protoplasmic rotation stopped, the chloroplasts shrunk to a very small size and gave out some chlorophyll to the surrounding water, and there was a very sharp rise in the exosmosis of chlorides from the cells. On return to water, respiration did not commence again and the cell became the prey of bacteria. The cell might recover, however, after lowering of the rate of photosynthesis. Cessation of respiration seemed to be the best criterion of death in Elodea, but if the cells did not all die at the same time, the death of some cells could not be detected by this means. It seemed that a cell

might live some time after protoplasmic rotation had ceased, but such cessation was an index that the lethal dose of anesthetic was approached, and it had the advantage of being observable in individual cells.

If the experiments in which protoplasmic rotation had ceased are omitted we may be confident that we are dealing with living cells and may summarize the data in the following table:

	PERCENTAGE CONCENTRATION	ABSORPTION OF O_2	ELIMINATION OF CO_2	EXOSMOSIS OF CHLORIDES	PROTOPLASMIC ROTATION	PHOTOSYNTHESIS	SIZE OF CHLOROPLASTS
Water		100	100	100	100	100	100
Alcohol	0.05			100		94	100
	1.00	112	105	100	100	75	100
	1.50	135	125	150	105	62	99
	3.00	189	140	500	135	44	79
	6.00	163	175	2000	71	00	52
Ether	1.50	150	117	200	90	49	76
	3.00	186	133	1000	30	12	68
Chloroform	0.05	113	100	1500	130	47	91
Chloretone	0.05	117	150	150	71	100	100

In the above table the results with 6% alcohol might best be omitted. Some shoots of Elodea seem to be injured by the alcohol and it seems probable that some dead cells may have been used in each experiment. Since rotation did not cease but was only retarded in the cells observed, these data are included to avoid criticism. In some cases rotation stopped in 3% ether.

It may be seen from the table that all the anesthetics caused increased oxygen consumption and the greater the concentration of the anesthetic the more effective it was. There seemed to be some delay in the elimination of CO_2 as shown by successive time periods in the same experiment. This factor and the lesser sensitiveness of the method may be the reason cause for the failure of exact agreement between the CO_2 and the O_2 data. With marked increase in respiration there was also marked increase in exosmosis of chlorides as determined with the nephelometer. Protoplasmic rotation might be increased or decreased by the anesthetic, whereas photosynthesis was always decreased. In higher concentrations of the anesthetic the size of the chloroplasts was markedly reduced.

Since the effect of anesthetics on the conscious and automatic (rhythmical) centers in the central nervous system has been so thoroughly studied, it was decided to omit such data. Cassiopeas of about 15 cm. diameter were deprived of their automatic nerve centers by cutting off the margin

of the bell. They were also deprived of the manubrium in order to eliminate the numerous mucous glands of that organ. The respiratory quotient was found to be about 95, and since the CO_2 data are less accurate than the oxygen data, only the latter are given. It was found that nerve conduction in the bell was abolished in one-half per cent ether. The jellyfish died at the end of an hour in 3% ether but by limiting the experiment to one hour, reliable data could be obtained at this concentration. Ether concentrations of one-half per cent, one per cent, two per cent and three per cent had no effect on the rate of respiration.

Owing to limited time it was thought best not to run through a long list of anesthetics in a hurried manner with no basis for hopes that a change in respiration rate might be observed, but to look for one which had been reported to change the rate. Carbon dioxide has been considered by many biologists as an anesthetic and used to anesthetize lower animals. I had previously observed that an increase in hydrogen ion concentration of the sea water might lower the respiration rate of Cassiopea, but had not determined whether this was due to H ions alone or to an associated increase in CO_2. The following experiments show that the metabolism is affected by CO_2 and not by increase in H ions without increase in undissociated CO_2 molecules.

Although Tortugas sea water contains about 44 cc. CO_2 per liter when estimated by adding acid and pumping it out, only a small fraction of this exists as CO_2 molecules in the water, most of it being in the form of HCO_3' and CO_3'' ions. If more CO_2 is added to the water a large increase in undissociated CO_2 molecules may be obtained. If HCl is added to the water, a limited increase in CO_2 molecules may be obtained, due to decomposition of carbonates and bicarbonates. An increase in H ions without increase in CO_2 molecules or even with decrease in CO_2 molecules may be obtained by adding enough HCl to neutralize the alkaline reserve, pumping out the CO_2 and adjusting the H ion concentration by minute additions of H_3PO_4. The results on relative rates of oxygen consumption after these methods of increasing H ions are as follows:

p_H	8.2	6.6	6.3	5.8	5.7	5.5
By adding CO_2	100	96	92	62	41	24
By adding HCl	100	98		70		
By adding HCl and removing CO_2	100			95		

In the above table the decrease of 5% in oxygen consumption after removing CO_2 is well within the limits of error of the method and is strikingly different from the decrease of 38% on adding CO_2 to obtain the same p_H without removal of CO_2. Therefore, the increased H ions are not responsible for the great fall in respiration after addition of CO_2.

In conclusion it seems evident that all anesthetics may not act in exactly the same way or exactly the same on both plants and animals, and

that, whereas anesthetics may induce surface changes assumed to be associated with permeability, they are active on the inside of the cell as evidenced in shrinkage of chloroplasts. Anesthetics affect different cell activities differently. This may be due to effects on different cell structures : plasma membrane, chloroplasts and cytoplasm.

A GENERAL SINKING OF SEA-LEVEL IN RECENT TIME

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Communicated by J. M. Clarke, March 10, 1920

Among the causes of world-wide or "eustatic" shifts of sea-level are: appropriate crustal movements whereby the volume of the ocean basin is changed; delta-building and volcanic extravasation on the sea-floor, the displacement of sea-water not being compensated by crustal subsidence; volcanic addition of new water to the ocean; subtraction of water which becomes chemically bound during the alteration of rocks; glaciation on land, lowering sea-level by the abstraction of water from the ocean; deglaciation on land, raising sea-level; changes in the earth's center of gravity and in her speed of rotation. Numerous as these possibilities are, eustatic shifts at definite times and in definite amounts have not often been postulated by geologists. The proof of such a shift, either positive or negative, is not easy. The present note records some field facts suggesting the probability of a sinking of general sea-level to the extent of nearly 20 feet, during the Human period. The suggestion is published, not to express a fixed conclusion but to invite criticism by those familiar with shorelines in different parts of the world, for its testing is manifestly a world-wide problem. The matter is important because it affects judgment as to the reality of many so-called uplifts of land or sea-bottom, and because a close study of the latest shift of sea-level may help to systematize the criteria for earlier and larger eustatic shifts.

Nearly twenty years ago the hypothesis of a recent, negative movement of sea-level first presented itself to the writer while engaged in the correlation of post-Glacial elevated strands in Labrador and Newfoundland with those of Quebec. The highest strand mapped between Newfoundland and Nachvak Bay, 500 miles to the northwest, was found to be strongly warped, like the highest strand from Newfoundland to Massachusetts. On the other hand, the lowest emerged terrace along the shores of the Gulf of St. Lawrence was seen to be conspicuously level throughout a distance of 300 miles or more below Quebec City. This apparent uniformity of level prompted the question whether this particular strand had been abandoned by the waves because of a sinking of general sea-level. For lack of accurate leveling, correlation with the more closely studied, lowest

beaches of Labrador was impossible, and with the data in hand serious belief in the hypothesis of a eustatic change was withheld.

However, that explanation was recalled by the discovery of new facts during recent field studies on the shores of New England, Florida, and the Samoan Islands. Meantime Goldthwait had made a careful study of the St. Lawrence strand, to which he gave the name "Micmac terrace;" the results of his leveling agree so well with those made in the other regions mentioned that the suspicion of past years became a hypothesis deserving more attentive consideration.

For the opportunity to collect data from the two tropical regions the writer is indebted to Dr. A. G. Mayor, Director of the Marine Laboratory of the Carnegie Institution of Washington.

In his excellent account of the Micmac terrace, Goldthwait noted the nearly uniform level of its inner edge at about 20 feet above high tide, as well as its relatively great strength and continuity, with width varying from a few feet to 1.5 miles or more. Though only a part of its width is due to wave-cutting and though the formations affected are weak, the higher level of the sea must have been steadily kept for a large fraction of post-Glacial time.[1] Farther east in the Gulf of St. Lawrence, on Anticosti, and again in Nova Scotia, Twenhofel has found wide terraces at about the same level.[2]

A similar feature characterizes the coast of Maine, as emphasized in the folios and monographs of the United States Geological Survey and as confirmed by the present writer.

Mayor had already found wave-cut rock benches, about 8 feet above high tide on all sides of Tutuila, the largest island of American Samoa.[3] The island is 16 miles long. At its eastern end is Aunuu island, and about 60 miles farther east is Tau island of the Manua group. The writer found very fine wave-cut benches at 8 to 10 feet above high tide on Aunuu and Tau, and others at the same general elevation on Olosega island, 7 miles west of Tau. The distance between the most widely separated benches is 75 miles; nowhere in that long stretch did the bench crests depart essentially from a constant level. Explanation by local uplift was at once seen to be highly improbable, for crustal uplift of such uniformity is unknown to geology.

Eight or ten feet of bench elevation does not, however, measure the total of recent emergence in Samoa. At several points in Tutuila large sea-caves were found. Their floors varied in height, from 14 or 15 feet at their mouths to 25 feet at the back walls of the caves. Many caves of similar forms and cut in similar rocks are being made by the surf of the present day. The lower lips of these newer caves are characteristically 4 to 6 or more feet below high-tide level and the floors rise inwards to heights of a few feet above high tide. Hence, the emerged caves were cut when the sea-level was nearly 20 feet higher than now. That assump-

tion also explains the 8-foot benches, for the cliffs now being cut on the Samoan headlands (where not protected by coral reefs) have regularly about 12 feet of water directly at their feet during high tide. The writer fully agrees with Mayor's conclusion that reef corals were not living on any of these Samoan shores when the emerged caves and benches were developed. Hence, the nearly or quite reefless headlands of the present time are those where the relation between sea-level and bench-level of the earlier period is best determined.

Correlation of the Samoan and North American terraces was suggested not only by the agreement in level but also by the state of preservation of the strand-marks, as well as by the comparable amounts of work done by the waves in the two regions during the higher stand of the sea.

The hypothesis of a eustatic shift must obviously meet the test of matching the facts observable on the shores of every continent and of most islands. The application of the test is difficult for many reasons which need not here be recounted, but a partial study of the records scattered through geological literature shows that the hypothesis merits discussion and further testing. The actual compilation of facts relates to the British Isles, the Atlantic coast of North America south of New York, the West Indies, Brazil, Patagonia, Antarctica, New Zealand, Australia, and many groups of the Pacific islands. For details the reader is referred to an article in a forthcoming number of the Geological Magazine (London).

A specially instructive example is seen in the agreement of the lower Cape May terrace of New Jersey and Pennsylvania, the Talbot terrace of Maryland (marine part), the Satilla terrace of Georgia, and the lower Pensacola terrace of Florida in age, height and strength of development, both among themselves and with the other terraces above-mentioned. The Pensacola-Satilla terrace alone keeps a practically uniform set of levels (maximum about 20 feet) for an air-line distance of 500 miles; the explanation of its emergence by crustal uplift seems hardly credible.

According to Andrews, "indications of the apparent elevation of the shore-line for a few feet are to be found along the whole eastern side of Australia;" he attributes the cause to "either an apparent rise of the land, or a retreat of the sea."[4] Süssmilch specifies the amount of the emergence (New South Wales) as "10 to 20 feet."[5] Cadell notes similarly recent emergence of western Australia by amounts between the same limits.[6] In this case nearly or quite uniform emergence has affected a thousand miles of coast on one side of a continent and several hundred miles on the other side, and again there is practical agreement with the amount of emergence shown in the mid-Pacific, in North America, and in many other regions.

Thus, in spite of complications, including the present-day warping of some coasts, the hypothesis appears to stand the test involving the amounts of local emergence.

A second test naturally inheres in the expectation that the deleveling should be synchronous in all parts of the ocean. Observers dwell on the recency of the sea-level shifts registered in all of the cases compiled. Fossils from the St. Lawrence, Scottish, Irish, Cuban, Patagonian, New Zealand, Australian, Funafuti, Murray Island, New Jersey, Maryland, Georgia, and Florida beaches, reefs, or coastal plains belong to species now living in the adjoining seas, or, exceptionally, to species now living in slightly warmer water. Great recency is also shown by the relatively small damage done by erosion to the strand-marks, except in the case of those exposed to powerful surf. The beaches of the higher latitudes are clearly post-Glacial and also of later dates than the post-Glacial uplifts following the melting of the ice-caps. Brögger puts the completion of the last 4-meter to 8-meter emergence of the Christiania region at about the year 500 B.C. The development of the corresponding sea-cliffs, benches, and other strand-marks is referred by him to the late Tapes (Neolithic) period, between 1400 B.C. and 2400 B.C. During late Tapes time the Christiania sea was about 2° C. warmer than now.[7] The fossils in the Patagonian beaches also betoken water warmer than the adjacent sea.

Simultaneous emergence of the many separate regions is the more credible because of the similarity in the histories of their respective coasts before emergence. Repeatedly one encounters the description of terrace, bench or beach near the 20-foot level as the best developed, the most conspicuous, of the most persistent in areas characterized by "raised" strand-marks. One may conclude that the different regions must have had sea-level nearly constant for a considerable time, during which the strand-marks were well incised or built up. Such widespread accordance itself must deepen suspicion that local deformations of the earth's crust have not been responsible for the recent emergence of so many continental and island coasts.

In conclusion, the facts at hand seem to permit belief in the synchrony of the different strand-markings and emergences here considered, but this second test of the hypothesis clearly demands further investigation.

Before the fact is established, it would be idle to dwell overmuch on the explanation of a 20-foot drop in the level of the ocean. The most promising idea appears to be that a few thousand years ago there was an increase in the volume of the existing, non-floating glaciers. If the Antarctic ice-cap were then thickened to the average amount of about 700 feet, an average sinking of sea-level to the extent of nearly 20 feet would be inevitable. In favor of this suggestion would be evidence of a world-wide oscillation of climate like that which seems to have affected the Christiania region in Recent time. If the whole earth was, in the Tapes period, a little warmer than now, less water may have been taken from the ocean to constitute the ice-caps; sea-level was a little higher than at present. The

oscillation as a whole would be but an incident in a series of climatic and oceanic changes which began with the opening of the Glacial period.

[1] Goldthwait, J. W., *Amer. J. Sci.*, **32**, 1911 (293).
[2] Twenhofel, W. H., *Ibid.*, **28**, 1909 (147); **30**, 1910 (66, 69).
[3] Mayor, A. G., These PROCEEDINGS, **3**, 1917 (523).
[4] Andrews, E. C., *New South Wales Handbook*, Brit. Assoc. Adv. Sci., 1914 (525, 532).
[5] Süssmilch, C. A., *Introduction to the Geology of New South Wales*, Sydney, 1911 (154).
[6] Cadell, H. M., *Trans. Edinburgh Geol. Soc.*, **7**, 1897 (179).
[7] Brögger, W. C., *Norges geol. Unders.*, No. 31, 1901 (713), and No. 41, 1905.

A NOTE ON THE HUMAN SEX RATIO

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Communicated by R. Pearl, March 20, 1920

From data collected at the Sloane Maternity Hospital in New York City, it has recently been shown[1] that there is a significantly higher male sex ratio at birth among progeny whose parent are not of similar nationality. This result confirmed a paper published by the Pearls[2] in 1908, based on data derived from vital statistics of the City of Buenos Ayres, and had in addition a decreased source of error due to improved methods of recording the data and the inclusion of still births as well as living births in the calculations.

During the past year, a further study of the same records has been made concerning the sex ratio among the progeny of certain other types of matings and a preliminary attempt at analysis made by contrasting the sex ratios of the offspring of *primipara* with those of subsequent births.

Table 1 shows the sex ratios of five categories of matings: European "pure," European "hybrid," United States white, British West Indies colored, and United States colored. European pure are matings in which both parents were of the same nationality. European hybrids are offspring whose parents are of different nationality, United States white include a random sample of parents coming from various parts of the United States but preponderantly from the East and especially from New York State and New York City. British West Indies colored, are offspring from colored parents born in the British West Indies, and United States colored are offspring from colored parents born anywhere in the United States.

In each case the probable error of the ratio obtained has been calculated for the numbers observed. On the basis of these probable errors, the differences between the various ratios can be fairly compared with respect to their probable significance.

As has before been shown, there is a significant difference between the European "pure" and the European "hybrid" and between the former

and the United States white. There is, however, no significant difference between the European "hybrid" and the United States white. This shows that in the data studied, the United States white ratio is essentially that of a hybrid race.

TABLE 1

	MALES	FEMALES	RATIO
(*a*) European Pure	2807	2689	104.54 ± 0.97
(*b*) European Hybrid	677	551	122.86 ± 2.14
(*c*) United States White	994	840	118.33 ± 1.71
(*d*) British W. I. Colored	667	618	107.92 ± 2.65
(*e*) United States Colored	695	723	96.12 ± 1.76

COMPARISON	DIFFERENCE	DIFFERENCE / PROBABLE ERROR OF DIFFERENCE
European Pure × European Hybrid	18.32 ± 2.35	7.8 Significant
European Pure × U. S. White	13.79 ± 1.9	7.2 Significant
European Pure × B. W. I. Colored	3.38 ± 2.82	1.2 Not significant
European Pure × U. S. Colored	8.42 ± 2.0	4.2 Significant
European Hybrid × U. S. White	4.53 ± 2.74	1.6 Not significant
U. S. White × U. S. Colored	22.21 ± 2.45	9.0 Significant
B. W. I. Colored × U. S. Colored	11.80 ± 3.18	3.7 Significant

This behavior of the United States matings is not in the least surprising for the number of different racial stocks involved in the production of the parents of the offspring recorded is undoubtedly large.

The British West Indies colored stock, while far from pure, is obviously more nearly so than the United States colored. This is true because of the fact that the United States colored in addition to including offspring whose grandparents were born in the United States, also admits the chance that part of the grandparents may have been themselves British West Indies stock, thus adding the hereditary variations of this stock to that of the United States colored stock. It is interesting to note that the ratio of the B. W. I. stock does not depart significantly from that of the European pure whites (see table 1). On the other hand, the United States colored differs significantly from both B. W. I. colored, and from European pure and United States whites.

We may, therefore, class our matings into two main groups—the European pure and the B. W. I. colored, as being relatively "pure" and the European hybrid, United States white, and United States colored as being relatively "hybrid."

Let us now examine the nature of the ratios when the progeny of *primipara* are separated from other births.

TABLE 2

	1ST BIRTHS			SUBSEQUENT BIRTHS				
	♂♂	♀♀	Ratio	♂♂	♀♀	Ratio	Difference	Difference / Probable Error of Diff.
European Pure	1236	1070	115.51 ± 1.5	1571	1614	97.33 ± 1.18	18.18 ± 1.86	9.7
B. W. I. Colored	313	302	103.64 ± 2.75	354	316	112.02 ± 2.76	8.38 ± 3.9	2.1
European Hybrid	303	250	121.20 ± 3.14	374	301	124.25 ± 2.91	3.05 ± 4.28	0.7
U. S. White	534	446	119.73 ± 2.26	460	394	116.75 ± 2.49	2.98 ± 3.36	0.9
U. S. Colored	264	282	93.61 ± 2.44	431	441	97.73 ± 2.03	4.12 ± 3.17	1.3

Table 2 shows that the three hybrid matings show a not significant difference between the sex ratio of offspring of *primipara* and those of subsequent births. On the other hand, there is a marked significance in the case of European pure matings and a barely possibly significant difference in the B. W. I. colored. The entire excess of male births in the total sex ratio of 104.54 in the European pure matings is found in the offspring of *primipara*—where 115.51 ± 1.50 males to 100 females are found.

It is interesting to note that the negro matings in every case differ from one another in exactly the opposite direction from the white races. Thus if the direction of arrows shows the change in the *male* ratio the following diagram may be constructed: Single arrows = white, double arrows = colored.

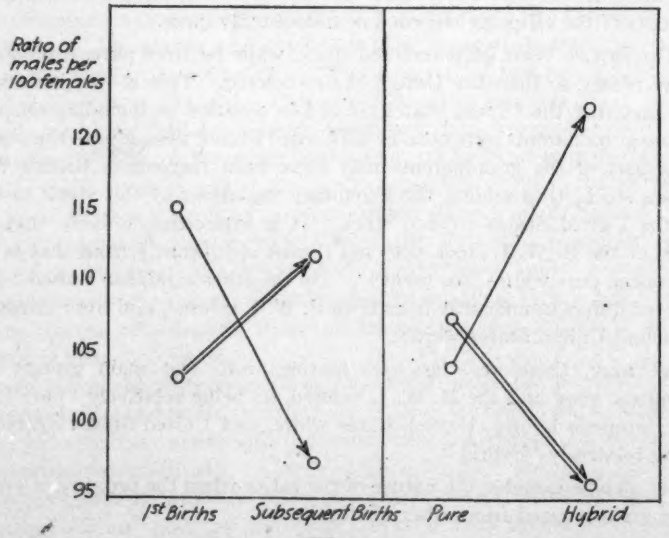

FIG. 1

The British West Indies colored race starting with a sex ratio of 103.64 ± 2.75 in the offspring of *primipara* shows in subsequent births a ratio of 112.02 ± 2.76 while in the European pure the ratio of first births is 115.51 ± 1.5 and subsequent births show a *decrease* of males; the ratio being 97.33 ± 1.18.

So also the differences between "pure" and "hybrid" races are in the opposite direction in the colored and white races. The "pure" European have a sex ratio of 104.54 ± 0.97 and the European hybrids a ratio of 122.86 ± 2.14—showing an *excess of males*. On the other hand the British West Indies colored which as we have seen is, compared with the United States colored, relatively the purer, has a ratio of 107.92 ± 2.65, while the United States colored has a ratio of 96.12 ± 1.76 an *excess of females*.

It seems, therefore, that the following conclusions as regards material in the records of the Sloane Maternity Hospital of New York City, may be drawn as a guide for further investigations.

(1) Hybrid white matings give a significant *excess of males* over "pure" white matings.

(2) Hybrid colored matings give a significant *excess of females* over relatively "pure" colored matings.

(3) The difference between the sex ratio of the United States born whites and the United States born colored is nine times its probable error.

(4) In "pure" European matings the offspring of *primipara* have a sex ratio of 115.51 ± 1.5, while the offspring from subsequent births have a ratio of 97.33 ± 1.18. The difference is 9.7 times its probable error.

(5) In the hybrid matings studied no such difference between the sex ratio of offspring of *primipara* and of subsequent births exists.

(6) The sex ratios of the United States white births recorded is not significantly different from that of *hybrid* European matings.

[1] *Proc. Soc. Exp. Biol. Med.*, 1919.

[2] *Biol. Bull.*, 1908.

THE EXISTENCE OF HOMOGENEOUS GROUPS OF LARGE IONS

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The Zeleny mobility apparatus has been modified by the author so as to be capable of giving a decisive answer to the question as to whether the large ions produced by spraying distilled water are of many different sizes or whether—as claimed by certain observers[1]—only a few different sizes are present in the ionization. In other words, is there a *continuous* spectrum of mobilities or a *line* spectrum? The investigation is fully described in an article which is about to appear in the *Physical Review*.

The mobility apparatus consists of a brass cylinder 160 cm. long and

5 cm. internal diameter, which is fitted with two co-axial electrodes 2 cm. in diameter and, respectively, 105 and 35 cm. long. (See fig. I.) The electrode L' is grounded, while L'' is connected to a pair of quadrants of an electrometer. Any desired potential may be established between the cylinder and the electrodes by means of a battery of dry cells.

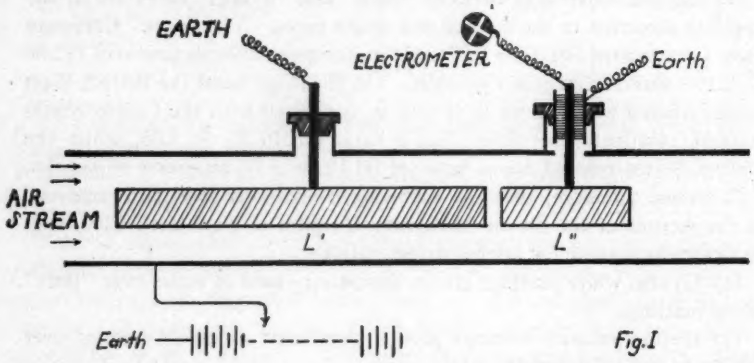


Fig. I

The ions to be tested are carried by a blast of air through the cylinder in a direction parallel to its axis. If there is no electric field, none of the ions are driven to the electrodes and the electrometer current is zero. When, however, a field is established and is increased progressively, more and more ions are driven from the air stream; the current increases and achieves its maximum value at a critical voltage V' at which all the ions are caught. As the field is still further augmented, the long, grounded electrode catches more ions, fewer reach the second electrode, and finally —at a voltage V''—the current again becomes zero. The voltages V' and V'', corresponding to the maximum and zero currents, respectively, may be computed for ions of mobility k by means of the formula:

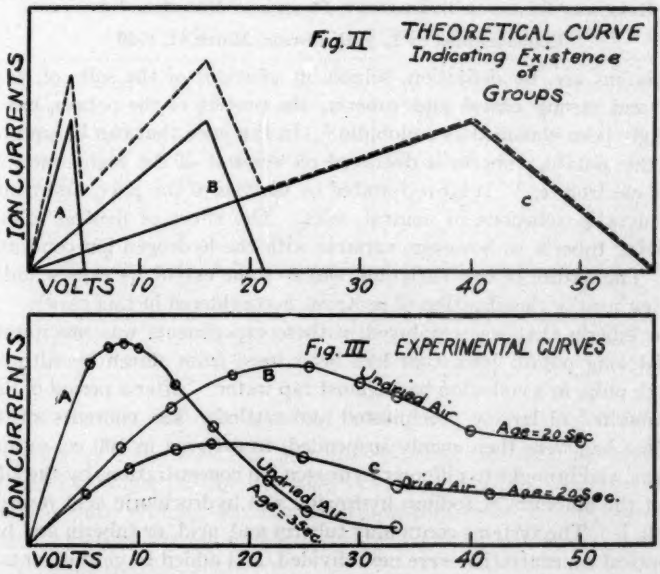
$$k = \frac{Q}{2\pi L'V''} \log_e b/a = \frac{Q}{2\pi(L' + L'')V'} \log_e b/a$$

a and b being the radii of the outer and inner cylinders, Q the volume of air traversing the tube each second, L' and L'' the lengths of the long and short electrodes.

Figure I shows the forms and relative positions of the three theoretical current-voltage curves for ions of respective mobilities, 0.0043, 0.0010, and 0.0004 cm. per second in a field of one volt per cm. These are the values found for successive groups of ions by the observers mentioned above.[1] The dotted line indicates the summation curve produced when the three classes of ions are simultaneously present in equal concentration. It will be noted that this curve has three well-defined *peaks*, each corresponding to a certain class of ions.

The experimental curves which have been determined by the author for ionization from spray, and also for that from hot wires, are all of the type shown in fig. III. The fact that no curve has a plurality of peaks proves that—contrary to the findings of other workers[1]—a series of widely separated, well-defined mobility groups does not exist.

Decrease of Mobility with Time.—It has been found that if the ions are tested soon after their production the mean mobility is relatively high. If, however, metal tubes are interposed between the sprayer and the testing tube, so as to increase the period between the production of the ions and their testing, the mean mobility decreases and higher voltages are



necessary to produce maximum ion currents. (Fig. III, (*A*) and (*B*).) The author, using a red hot platinum wire as a source of ionization, finds that the rate of decrease of mobility can be retarded by drying the air in which the ions are produced. (Compare curves (*B*) and (*C*), fig. III.) This indicates that the decrease of mobility is due, in part at least, to the growth of the ions by condensation of water vapor.

Nature of the Large Ion.—It has now been shown that—contrary to the findings of other observers[1]—a series of widely separated, well-defined mobility groups does not exist in the ionization from spray. Their hypothesis that the large ion is a complex cluster of stable water globules may consequently be abandoned in favor of the simpler conception of J. J. Thomson and others who have pictured the large ion as merely a charged

globule of water having a radius of equilibrium which is a function of the humidity of the air.

[1] McClelland, J. A. and Nolan, P. J., *Proc. Roy. Irish Acad.*, A 33, 1916 (29); Nolan, P. J., *Ibid.*, A 33, 1916 (10); McClelland, J. A. and Nolan, P. J., *Ibid.*, A 35, 1919 (1).

THE RELATION BETWEEN THE ISOELECTRIC POINT OF A GLOBULIN AND ITS SOLUBILITY AND ACID COMBINING CAPACITY IN SALT SOLUTION

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Globulins are, by definition, soluble in solutions of the salts of strong acids and strong bases[1] and tuberin, the protein of the potato, has accordingly been classified as a globulin.[2] In the juice that can be squeezed from the potato, tuberin is dissolved on account of the high concentration of electrolytes.[3] It is precipitated by dialysis of the juice, and readily redissolves in solutions of neutral salts. The effect of neutral salts in dissolving tuberin is, however, variable with the hydrogen ion concentration. The nature of this variation, and to some extent its causes and its meaning for the classification of proteins, is considered in this paper.

The tuberin that was employed in these experiments was precipitated by dialyzing potato juice that had been freed from starch by filtration through pulp, in a collodion bag against tap water. After a period of days the tuberin had largely precipitated and settled. The contents of the collodion bag were then evenly suspended, transferred in 100 cc. samples to flasks, and brought to different hydrogen ion concentrations by the addition of the amounts of sodium hydroxide and hydrochloric acid recorded in table I. The systems containing tuberin and acid, or tuberin and base in identical concentration were next divided, and added to vessels containing different amounts of sodium chloride. The systems, therefore, varied in two respects; in the first place in the amount of free, and, therefore, of combined protein, acid or base; in the second place in the amount of salt that they contained. After they had stood at constant temperature for about 6 hours, and it was believed that equilibrium had been established, the hydrogen ion concentrations of the systems were electrometrically determined, and the solubility of the tuberin in them was estimated by determining nitrogen in an aliquot part after the precipitated tuberin had been removed by centrifuging. In certain of the systems it will be noted that all of the protein dissolved. In these cases, therefore, the data do not represent solubility; that is solutions saturated with respect to protein.

The results obtained in this preliminary investigation were not, it is true, altogether regular. Abnormal values of nitrogen and also of hydro-

gen ion concentration appear in the data. None the less, certain conclusions can quite safely be drawn, the more so since they are borne out by several other experiments.

TABLE 1

EFFECT OF NaCl UPON SOLUBILITY AND UPON ACID COMBINING CAPACITY OF DIALYZED TUBERIN

N/10 HCl added to 100 cc. dialyzed potato protein cc.	NaCl ADDED TO 100 CC. OF DIALYSED POTATO PROTEIN. VOLUME INCREASED BY ADDED ELECTROLYTES TO 120 CC.											
	0 gm.	0.25 gm.	0.5 gm.	1.0 gm.	2.0 gm.	5.0 mg.	0 gm.	0.25 gm.	0.5 gm.	1.0 gm.	2.0 gm.	5.0 gm.
	p_H						Tuberin dissolved (mg. nitrogen in 10 cc.)					
50	1.58	1.58	1.58		1.56	1.46	5.96	5.79	5.79	5.79	5.70	3.40
20	2.67						5.70	5.70	5.45	5.11	4.34	2.55
5	3.28	3.33	3.37	3.50	3.49	3.45	5.45	5.28	5.34	4.94	4.09	3.05
3	3.93	3.97	4.02	4.09	4.03	4.13	4.77	5.28	4.17	4.34	4.68	4.17
2	4.29	4.34	4.41	4.44		4.34	4.09	4.17	4.51	4.09		4.26
1	5.22	5.13	5.13				3.40	3.83	4.09	4.43	4.68	4.60
0	5.71	5.68	5.92	5.75	5.48	5.36	2.47	4.09	3.66	5.02	4.09	4.26
NaOH.												
0.5	6.40	6.23	6.02	6.28	6.10	5.84	3.58	4.51	4.85	5.19	4.77	4.94
1	6.95	6.76	6.78			6.52	4.98	4.17	4.77	5.96	5.19	5.36
3	8.99	8.77	8.71	8.77	7.95		5.87	5.02	5.21	5.70	5.87	5.87
5	9.41	9.06	9.23	9.25	9.09	8.98	5.87	5.70	5.87	5.62	5.87	5.70
10	10.39	10.09	10.03	10.20	9.72	9.83	5.96	6.04	5.79	5.70	5.87	5.79

I. THE EFFECT OF SODIUM CHLORIDE UPON THE SOLUBILITY OF TUBERIN

Solubility of Tuberin in Salt Solution.—The tuberin that had been precipitated by dialysis, in the way that has been described, must not be regarded as pure protein, but rather as a natural protein compound. It contained phosphorus and may have contained calcium. It was, however, largely redissolved by salt.

The solubility of the tuberin was increased by the addition of sodium chloride to the systems that were otherwise unchanged. It was increased more by sodium chloride in the systems which contained small amounts of sodium hydroxide. However, when a small amount of acid was added the power of sodium chloride to increase the solubility was greatly weakened. This "effect of minute quantities of acid on the solubility of a globulin in salt solutions" appears to be quite general. It was studied by Osborne and Campbell in 1897[4] and will be discussed later in this paper.

In our experiments, which were extended over a wider range of hydrogen ion concentration, the effect of sodium chloride steadily decreased as the acidity increased, until at hydrogen ion concentrations in the neighborhood of 10^{-4} N, the solubility of the protein was nearly independent of the salt concentration.

Solubility of Tuberin in Acid Solution.—Not only was tuberin dissolved by salt solutions, in the absence of acids, but it was also dissolved by acid in the absence of salt. In solutions of lower hydrogen ion concentration than 10^{-4} N, the dialyzed tuberin compound was only slightly soluble;* less so than in salt solutions. At greater hydrogen ion concentrations than 10^{-4} N, tuberin was largely dissolved in the systems to which no sodium chloride had been added. Its solubility in solutions of this degree of acidity was, however, decreased by sodium chloride. Indeed in the presence of sufficient salt the tuberin was nearly completely precipitated. The precipitation of many other globulins besides tuberin from acid solution by neutral salts has been observed. Osborne[5] studied the precipitation of edestin under these circumstances, and Hardy[6] that of serum globulin. This phenomenon is also apparently quite general.

Solubility of Tuberin at pH 4.3 Independent of Salt.—Between the systems of such acidity that sodium chloride decreased the solubility of tuberin and the more nearly neutral ones in which sodium chloride increased solubility, there existed a narrow range where sodium chloride had very little effect upon the solubility of tuberin. This range coincided with a slightly lower hydrogen ion concentration than 10^{-4} N.

These results are reported in table I. If represented graphically, the solubility of tuberin plotted as ordinates, and hydrogen ion concentration as abscissae, the curves representing the solubility of tuberin in the presence of different amounts of sodium chloride converge, and intersect each other within a narrow range of hydrogen ion concentrations, slightly lower than 10^{-4} N, probably nearest to 0.5×10^{-4} (pH 4.3).

The Isoelectric Point of Tuberin.—In a previous investigation the method of cataphoresis was employed in determining "the Isoelectric Points of the Proteins in Certain Vegetable Juices."[3] Potato juice was brought to different hydrogen ion concentrations and the migration of tuberin in an electric field determined. The direction of migration was found to change at a hydrogen ion concentration slightly lower than 10^{-4} N. At greater acidities tuberin migrated to the cathode, at more nearly neutral reactions to the anode. At hydrogen ion concentrations slightly lower than 10^{-4} N, tuberin migrated in neither direction. At this reaction, therefore, it was isoelectric.

The Ionization of Tuberin.—The ionization of a protein compound can be inferred from the direction of its migration during cataphoresis. In neutral potato juice and potato juice less acid than 10^{-4} N, tuberin bore a negative charge. Presumably therefore tuberin as it existed in the juice of the potato was ionized as an acid. This compound decomposed on the addition of the stronger acid HCl, and tuberin was freed at its isoelectric point. In the neighborhood of its isoelectric point tuberin was, moreover, less ionized than at greater or at lesser acidities and was also

* This requires further study.

combined with fewer of the electrolytes that existed in potato juice or were formed by the addition of acid to it. For this reason the flow of current during cataphoresis was greatest at the isoelectric point (3, p. 155). At hydrogen ion concentrations greater than the isoelectric point, where tuberin migrated to the cathode, and bore, therefore, a positive charge, it was evidently ionized as base and combined with acid.

Solubility of Acid and of Basic Tuberin Compounds.—The coincidence of the hydrogen ion concentration at which sodium chloride has least effect upon the solubility of tuberin with the isoelectric point allows of but one interpretation; sodium chloride affects tuberin differently as the migration of the protein varies. At hydrogen ion concentrations greater than 10^{-4} N, where tuberin was ionized as base and combined with acid, sodium chloride decreased its solubility. When tuberin was least ionized, at its isoelectric point, sodium chloride had least effect upon its solubility. Expecially when tuberin was ionized as acid did sodium chloride increase its solubility in the way that has led to its being classified as a globulin.

Solubility of Other Globulins.—This conception of the behavior of a globulin is not new. It was expounded by W. B. Hardy in his Croonian Lecture of 1905[6] on the basis of his studies of serum globulin.[7] Excepting in one point, which will presently be considered, his observations and conclusions agree with ours on tuberin. Neutral salts only increased the solubility of serum globulin at reactions more nearly neutral than its isoelectric point. At acidities greater than the isoelectric point, neutral salts precipitated serum globulin, as they did tuberin.

The earlier observations that have been mentioned indicate (1) that the solubility of many other globulins in salt solutions is decreased by small amounts of acid, and (2) that many globulins are precipitated from acid solution by small amounts of neutral salt. Up to the present the isoelectric points of most of these proteins have not been determined.* The way in which they are ionized is, however, suggested by these criteria. This appears from a consideration of the effect of sodium chloride upon the dissociation of the compounds that tuberin forms at greater and at lesser acidities than correspond to its isoelectric point.

II. THE EFFECT OF SODIUM CHLORIDE UPON THE ACID COMBINING CAPACITY OF TUBERIN

The chemical analysis of the effect of sodium chloride upon the ionization of the different tuberin compounds, whose solubility it differently affects, is carried one step further by the measurements of hydrogen ion concentration. The hydrogen ion concentration of a system containing protein and acid or protein and base is, as a first approximation, defined by the ratio of protein to acid, or protein to base,[9,10,11] and depends merely

* Rona and Michaelis[8] give the isoelectric point of serum globulin as 0.36×10^{-5} and of edestin as 1.3×10^{-7}. These values depend in part, however, upon the points of maximum precipitation of the proteins.

on the ionization constant of the acid or base and the amphoteric constants of the protein. The effect of a neutral salt upon such systems has recently been exhaustively studied by Sörensen, Hoyrup, Hempel and Palitzsch.[9] It is illustrated by the data in table I. These are in substantial agreement with those recently published by Sörensen and his collaborators. They moreover derived an equation from the mass law which enabled them to calculate the hydrogen ion concentration of any ampholyte in the presence of an acid, and the salt of the acid and a strong base, if the dissociation constants of ampholyte, acid, and salt, and the concentrations of the components of the systems were known. In this equation "the influence of the salt only appears by the fact that the values of" "the degree of dissociation of the acid" and "of the salt of the acid together with the ampholyte" "vary with the concentration of the salt." (9, pp. 93 and 101.) In other words, the effect of a neutral salt with a common ion is to depress the dissociation of the acid, and of the salt of the acid with the protein. As a result the acid combining capacity of the protein is increased, and the hydrogen ion concentration decreased. If the protein is ionized as acid on the other side of its isoelectric point and is combined with base, the depression in the dissociation of the base, and of the salt of the protein and the base, causes the hydrogen ion concentration to increase. The effect of salt in depressing the dissociation of the protein compound, however ionized, thus causes the hydrogen ion concentration of protein systems to converge upon the isoelectric point of the protein. As a result Sörensen has concluded:

"at hydrogen-ion concentrations which are not situated at or in the neighborhood of the isoelectric point of the ampholyte the capacity to combine with acids

"(*a*) is independent of the ampholyte concentration;

"(*b*) increases with rising concentration of salt;

"(*c*) is positive at hydrogen-ion concentrations superior to that corresponding with the isoelectric reaction of the ampholyte, and

"(*d*) is negative—that is, the ampholyte is combined with surplus base—at hydrogen-ion concentrations inferior to that corresponding with the isoelectric point of the ampholyte.

"At hydrogen-ion concentrations corresponding with or situated in the neighborhood of the isoelectric point of the ampholyte, the conditions are not easily discernible; the capacity to combine with acids is in this case not independent of the concentration of the ampholyte, but is the greater the smaller the latter is." (9, p. 161.)

Whenever complicating colloidal phenomena do not interfere, therefore, the mass law demands that a neutral salt increase the hydrogen ion concentration of systems containing protein when ionized as acid, and decrease the hydrogen ion concentration when ionized as base. Salt should, accordingly, have least effect when the hydrogen ion concentration of the

system coincides with the isoelectric point of the protein. Sörensen's studies, therefore, add another property—acid combining capacity—in which the singular point in protein behavior coincides with the isoelectric point, and suggests another method which, in the absence of complicating phenomena, may be used to determine the isoelectric point.

The only apparent effects of sodium chloride upon the hydrogen ion concentration of systems containing tuberin and sodium hydroxide, or tuberin and hydrochloric acid, follow from this theory.* When sodium chloride was added to such systems the acid binding capacity was increased, and the hydrogen ion concentration decreased, if tuberin was ionized as base at acidities greater than the isoelectric point. The hydrogen ion concentration was increased at more nearly neutral reactions. The hydrogen ion concentration of the system whose acid binding capacity was least affected by the addition of sodium chloride was slightly lower than 10^{-4} N, and, therefore, in the neighborhood of the isoelectric point of tuberin.

Dissociation of Tuberin in Salt Solution.—The increased hydrogen ion concentration of tuberin systems less acid than pH 4.3 to which sodium chloride was added shows that the dissociation of the tuberin compound was depressed, and that as a result tuberin combined with surplus base (the amount of base bound by tuberin, and by depression of the dissociation of all electrolytes being measured by the increase in free acid). We have seen that under these circumstances the solubility of tuberin increased.**

Tuberin also combined with base in the systems to which sodium hydroxide was added. An increase in solubility also followed the formation of this tuberin compoound, which may have been similar to that formed by sodium chloride. Tuberin never dissolved as completely, however, in neutral solutions to which no sodium chloride was added. Apparently this compound of tuberin is more soluble when little dissociated. That its dissociation products—like those of many other globulins which appear upon dialysis or dilution of their solutions—are very insoluble, is certain.

Small amounts of hydrochloric acid decomposed the compounds that were formed by the more weakly ionized acid, tuberin, at reactions less acid than its isoelectric point. We have seen that sodium chloride and sodium hydroxide favored the formation of this tuberin compound, and, therefore, increased its solubility. By decomposing it hydrochloric acid decreased the solubility of tuberin in salt solutions.

The observations of Osborne, that have already been referred to, indicate that the decreased solubility of many other globulins in salt solutions, to which small amounts of acid had been added, resulted from their acid

* It is immaterial whether the change of hydrogen ion concentration caused or was caused by the change in state of the protein.

** The tuberin preparation was probably not a simple sodium compound. It contained phosphorus, and may have contained calcium. The increase in solubility may, therefore, in part, have been associated with change in the protein compound.

isoelectric points, and the decomposition of their compounds with bases. If the isoelectric points of these globulins had not been acid, but had occurred at an alkaline reaction, their acid compounds would have been decomposed by bases, and as a result they would probably have been rendered less soluble by bases rather than by acids.

Dissociation of Tuberin in Acid Solution.—In the presence of hydrogen ion concentrations greater than the isoelectric point, tuberin ionized as a base and dissolved as an acid compound. The effect of sodium chloride in precipitating tuberin from such solutions is perhaps less clear. Hardy studied the identical effect upon serum globulin. He believed that proteins formed addition products with neutral salts through their amino groups, and that under these circumstances neutral salts displaced acid from combination with protein. If that were so the acidity of such systems would increase. We have seen that in the case of tuberin quite the contrary occurs. Tuberin chloride binds more rather than less acid in the presence of sodium chloride. For the present, therefore, it is simpler merely to assume that tuberin chloride is little soluble when little dissociated.

Classification of Tuberin as a Globulin.—The classification of tuberin as a globulin depends, as our observations indicate, upon the solubility in salt solutions of the compounds that tuberin forms when ionized as an acid, and upon the insolubility of the products of the hydrolytic dissociation of these same compounds. It would appear from Osborne's observations upon the behavior of many other globulins toward small amounts of acid, that they too have acid isoelectric points, and are, therefore, ionized as acids in neutral salt solutions. If the isoelectric points of these proteins had been alkaline, their solubility in salt solution would have been decreased by bases rather than by acids. But whether the relations that have been described are characteristic of all globulins, or merely of tuberin, it appears from them how intimately the classification of proteins according to solubility involves their isoelectric points.

I am greatly indebted to the Director of the Harriman Research Laboratory, the Roosevelt Hospital, New York, for courteously extending to me the facilities of his laboratory after the research that I had been conducting there for the Surgeon General's Office was concluded. This enabled me to consider these more theoretical aspects of the earlier problem.

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[6] Hardy, W. B., *Proc. Roy. Soc., London* (B), **79**, 1907 (413–426).

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[11] Loeb, J., *Ibid.*, **1**, 1918–19 (237).

THE CARBON DIOXIDE: HEAT RATIO IN CATTLE

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Communicated by F. G. Benedict, March 24, 1920

It was recently suggested to us by Dr. F. G. Benedict that if the ratio of the carbon dioxide excreted to the heat produced by cattle should prove to be sufficiently constant, this fact might be utilized as the basis for determinations of the energy metabolism of this species which would be approximately correct and which would require only a comparatively simple technique. In view of the great value which such a simplified method would have, especially for agricultural investigators, and of the further fact that a new form of respiration apparatus designed to be used in this manner has been constructed by Benedict in coöperation with the New Hampshire Agricultural Experiment Station, we have thought it worth while to study the results of our calorimetric experiments on cattle from this point of view and we present the results of that study herewith.[1]

These experiments were made with the Atwater-Rosa respiration calorimeter which has been in use here since 1901, the heat production as well as the elimination of carbon dioxide being measured, and are, so far as we are aware, the only direct determinations of the heat production of this species. The measured heat production has been compared with the carbon dioxide elimination in 188 24-hour periods.[2] The carbon dioxide included, of course, the considerable amount arising from the methane fermentation in the rumen. The following results were obtained for the heat expressed in kilogram calories divided by the carbon dioxide expressed in grams.

CARBON DIOXIDE : HEAT RATIO OF CATTLE

Range	2.1500 to 3.1500
Mean value	2.4947 ± 0.0085
Standard deviation	0.1713 ± 0.0060
Median	2.4938
Theoretical mode	2.4974
Error of single variate	±0.12
Coefficient of variability	6.87%

In 51 of these periods we have also data for the carbon dioxide and heat production of the animals in the standing and lying positions respec-

tively. A comparison of these shows a somewhat smaller ratio in the lying than in the standing position.

CARBON DIOXIDE : HEAT RATIO OF CATTLE

	STANDING	LYING
Range	1.8600 to 2.9400	1.6900 to 2.5700
Mean value	2.5129 ± 0.0185	2.2529 ± 0.0149
Standard deviation	0.196 ± 0.013	0.158 ± 0.011
Median	2.5020	2.2713
Theoretical mode	2.4802	2.3081
Error of single variate	±0.13	±0.11
Coefficient of variability	7.80%	7.01%

A further study of the results indicated that the rather wide range in the single values of the CO_2 : heat ratio was caused in large degree by variations in the amount of feed consumed, the ratio tending to decrease as the amount of feed increased. This is what would have been expected for two reasons. First, the CO_2 : heat ratio for the methane fermentation, although not very satisfactorily determined, is undoubtedly much lower than that due to the tissue metabolism of the animal and as the feed is increased this constitutes an increasing proportion of the total elimination. Second, it is clear that the formation of fat from carbohydrates which takes place on a heavy ration would tend to lower the ratio; while, on the other hand, the oxidation of body fat on an insufficient ration would tend to increase the ratio. It should be noted also that our subjects were performing no external work and while not absolutely at rest were for the most part standing or lying quietly. Any considerable muscular exertion would doubtless have materially increased the ratio.

In 99 48-hour experiments, the factor of the varying size of the subjects being eliminated by computing all the results per kilogram of live weight, it appeared from a graph in which the abscissas represented the total weights of feed consumed and the ordinates, respectively, the grams of carbon dioxide excreted, the calories of heat measured and the ratio Heat/CO_2, that the individual results grouped themselves quite closely about three straight lines which could be represented by the three following linear equations, in which

x = Air-dry weight of feed in grams per kilogram of live weight
y_1 = Calories of measured heat per kilogram live weight
y_2 = Grams of CO_2 per kilogram live weight

$$y_3 = \frac{\text{Heat}}{CO_2} = \frac{y_1}{y_2}$$

(1) $y_1 = 0.869x + 14.176$
(2) $y_2 = 0.455x + 4.365$
(3) $y_3 = -0.0226x + 2.802.$

A computation of the closeness of fit of the lines represented by the foregoing equations to the individual results gives the following values for the index (Δ) and the probability (P).

$$\Delta_1 = 4.489 \qquad P_1 = 0.9508$$
$$\Delta_2 = 2.467 \qquad P_2 = 0.9640$$
$$\Delta_3 = 0.663 \qquad P_3 = 0.9986$$

It would perhaps have been preferable to make the comparisons with the dry matter of the feed consumed. No succulent feeds, however, were contained in the rations and it does not seem probable that the slight differences in moisture content can have materially affected the conclusions. We have not attempted to distinguish between the effects of different kinds or classes of feeding stuffs and doubt whether our data are sufficiently extensive to warrant it.

It would appear from the foregoing data that within the range of these experiments, i. e., with feed varying from 5 to 27 grams per kilogram live weight, equation (3) may be used for computing the CO_2 : heat ratio of cattle when their live weight and the amount of feed consumed are known, and that by the use of this ratio the heat production may be computed from the observed CO_2 production with a good degree of accuracy, at least for animals on dry feed and not performing work.

It should be noted, however, that the values of y_3 computed from equation (3) differ slightly from the ratios computed from the corresponding values of y_1 and y_2, indicating that y_3 is not a strictly linear function of x. The discrepancy does not appear sufficient to affect materially the computation of the heat production from the CO_2 on the amounts of feed used in these experiments but for the fasting animal, i. e., when $x = 0$, the discrepancy is much more considerable, viz.:

$$\frac{y_1}{y_2} = 3.25 \qquad y_3 = 2.80$$

It appears probable that the values of y_3 would be represented more exactly by a line slightly convex toward the horizontal axis and curving somewhat more sharply upward as the value of x approaches zero, and that for very small amounts of feed the CO_2 : heat ratio computed from the values of y_1 and y_2 is more accurate than that computed from equation (3). This conclusion is strengthened by the fact that the fasting value thus obtained approximates quite closely that observed by Benedict in his experiments on fasting men.

[1] The entire credit for the mathematical discussion of the results as well as for the laborious computations involved is due to Mr. Braman.

[2] In most instances two of these periods constituted the two halves of one of the 48-hour experiments mentioned subsequently.

ON KUMMER'S MEMOIR OF 1857 CONCERNING FERMAT'S LAST THEOREM

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Communicated by L. E. Dickson, March 6, 1920

The article referred to in the title appeared in the *Mathematische Abhandlungen* of the Berlin Academy for the year 1857, pp. 41–74. In the present note it will be pointed out that the proofs of the results given by Kummer are inaccurate and incomplete in several respects. The discussion will be confined exclusively to the indication of those parts of the argument which are deficient or incorrect, and in no case will methods for correcting the proofs or supplying the missing analysis be considered here.

In the first place Kummer divides his work, as usual, into the discussion of the two cases arising from

$$x^\lambda + y^\lambda + z^\lambda = 0 \qquad (1)$$

where x, y and z are rational integers and λ is an odd prime. The *first case* arises in the consideration of (1) when xyz is prime to λ, and the *second case* when xyz is divisible by λ.

For the first case he shows that if (1) is satisfied then $B_{(\lambda-3)/2}$ and $B_{(\lambda-5)/2}$ are each divisible by λ, the B's being Bernoullian numbers,

$$B_1 = 1/6,\ B_2 = 1/30, \text{ etc.}$$

The proof is given on pages 61–65 of the article in question. No criticisms will be made here of this part of the work.

For the second case Kummer concludes that the relation (1) is impossible under the three assumptions:

1. *The first factor of the class number H is divisible by λ but not by λ^2*

2. *If $B_\nu \equiv 0 \pmod{\lambda}$, $\nu < 1/2(\lambda-1)$, there exists an ideal in the field $\Omega(\alpha)$, $\alpha^\lambda = 1$, $\alpha \neq 1$, with respect to which as a modulus the unit*

$$E_\nu(\alpha) = \prod_{k=0}^{\mu-1} e(\alpha^{\gamma^k})^{\gamma^{-2k\nu}}$$

is not congruent to the λth power of an integer in $\Omega(\alpha)$.

3. *The Bernoullian number $B_{\nu\lambda}$ is not divisible by λ^3.*

In the above assumptions $H = h_1 h_2$, where

$$h_1 = \frac{P}{(2\lambda)^{\mu-1}},\ h_2 = \frac{D}{\Delta},\ \mu = \frac{\lambda-1}{2},$$

and P, D and Δ are defined as follows:

Let β be a primitive root of $\beta^{\lambda-1} = 1$ and λ a primitive root of λ. Then

$$P = \prod_{j=1}^{\mu} \phi(\beta^{2j-1}),\ \phi(\beta) = 1 + \gamma_1\beta + \gamma_2\beta^2 + \ldots + \gamma_{\lambda-2}\beta^{\lambda-2}$$

where γ_i is the least positive residue of γ^i modulo λ. Also

$$e(\alpha) = \sqrt{\frac{(1-\alpha^\gamma)(1-\alpha^{-\gamma})}{(1-\alpha)(1-\alpha^{-1})}},$$

and if lx denotes the real value of $\log x$, then

$$D = \begin{vmatrix} le(\alpha), & le(\alpha^{\gamma}) \ldots\ldots\ldots le(\alpha^{\gamma^{\mu}-2}) \\ ie(\alpha^{\gamma}), & le(\alpha^{\gamma^2}) \ldots\ldots\ldots le(\alpha^{\gamma^{\mu}-1}) \\ & \ldots\ldots\ldots\ldots \\ & \ldots\ldots\ldots\ldots \\ le(\alpha^{\gamma^{\mu}-2}), & le(\alpha^{\gamma^{\mu}-1}) \ldots\ldots le(\alpha^{\gamma^{2\mu}-4}) \end{vmatrix}.$$

If $\epsilon_1(\alpha), \epsilon_2(\alpha), \ldots\ldots \epsilon_{\mu-1}(\alpha)$ are fundamental units in $\Omega(\alpha)$, that is, units which have the property that all units in the field can be expressed as products of powers of them multiplied by $\pm\alpha^m$, then

$$\Delta = \begin{vmatrix} l\epsilon_1(\alpha) \ldots\ldots\ldots\ldots l\epsilon_{\mu-1}(\alpha) \\ \ldots\ldots\ldots\ldots \\ \ldots\ldots\ldots\ldots \\ l\epsilon_1(\alpha^{\gamma^{\mu}-2}) \ldots\ldots l\epsilon_{\mu-1}(\alpha^{\gamma_{\mu}-2}) \end{vmatrix}.$$

To establish the above result Kummer essays to prove four direct theorems in the theory of the field $\Omega(\alpha)$. These theorems are set forth later in this note and numbered I to IV. As will be shown, the proofs of theorems I and IV are incomplete as several unproved statements are made and the proofs of II and III are inaccurate, since use is made of a formula which is not true in general.

In *Crelle*, **44**, 1852 (134), Kummer gave the relation

$$(\lambda - 1)l\left(\frac{(\varphi(\alpha)}{\varphi(1)}\right) \equiv l\left(\frac{N\varphi(\alpha)}{[\varphi(1)]^{\lambda-1}}\right) + \sum_{i=0}^{\lambda-2} \frac{d_o^{i\lambda^n} l\varphi(e^v)}{dv^{i\lambda^n}} X_i(\alpha) \qquad (2)$$

modulo λ^{n+1}. In this formula $\varphi(\alpha)$ is an algebraic integer in the field $\Omega(\alpha)$, and the symbol

$$l\left(\frac{\varphi(\alpha)}{\varphi(1)}\right)$$

modulo λ^{n+1}, represents that part of the logarithmic series

$$\frac{\varphi(\alpha) - \varphi(1)}{\varphi(1)} - \frac{1}{2}\left(\frac{\varphi(\alpha) - \varphi(1)}{\varphi(1)}\right)^2 + \frac{1}{3}\left(\frac{(\varphi(\alpha) - \varphi(1)}{\varphi(1)}\right)^3 \ldots\ldots$$

none of whose terms are divisible by λ^{n+1}. The symbol $N\varphi(\alpha)$ stands for the norm of $\varphi(\alpha)$ and

$$\frac{d_o^s l\varphi(e^v)}{dv^s}$$

indicates that $\log \varphi(e^v)$ is to be differentiated s times and zero substituted for v in the result. Further,

$$X_i(\alpha) = \alpha + \gamma^{-i\lambda^n}\alpha^{\lambda} + \gamma^{2i\lambda^n}\alpha^{\gamma^2} + \ldots\ldots + \gamma^{-(\lambda-2)i\lambda^n}\alpha^{\gamma^{\lambda-2}}.$$

F. Mertens (*Wiener Berichte*, **126**, 1917, Abt. IIa, pp. 1337–43) points out that the relation (2) is not true in all cases and shows that an error occurs in Kummer's argument on page 133 of the latter's paper.

We shall now examine Kummer's 1857 memoir in detail and in the light of what has just been mentioned.

Under assumption 1, given above, namely, that h_1 is isdivible by λ but not by λ^2, Kummer proceeds to show that only one of the numbers B_i, $i = 1, 2, \ldots\ldots, \mu - 1$, is divisible by λ. In this connection he states (p. 42, 5th line from bottom) "....denn der erste Faktor der Klassenanzahl muss, wie aus meiner Untersuchung der Teilbarkeit der Klassenanzahl durch λ (*Liouville's Journal*, **16**, 1851 (473)) unmittelbar folgt, den Faktor λ mindestens so viel mal enthalten, als wie viele der $(\lambda - 3)/2$ Bernoullischen Zahlen durch λ theilbar sind." On examining the paper referred to by Kummer we find that he reduces h_1 modulo λ and obtains a residue which may be expressed in the form

$$m\prod_{i=1}^{\mu-1} B_i$$

where m is an integer prime to λ. At no place in this work however, does he refer to any reduction of h_1, modulo λ^n, $n>1$. Hence, he is not warranted in making, without additional argument, the statement given above.

Having concluded that only one of the first $\mu - 1$ Bernoullian numbers is divisible by λ, Kummer calls this one B_ν and derives (p. 44), under assumption 1, the following:

Theorem I. *If* $B_\nu \equiv 0 \pmod{\lambda}$, *and* $h_2 \equiv 0 \pmod{\lambda}$ *then* $E_\nu(\alpha)$ *is the* λ^n *power of a unit in* $\Omega(\alpha)$.

On page 45 an attempt is made to prove, under assumption 1, the

Theorem II. *If* $B_\nu \equiv 0 \pmod{\lambda}$ *but* $B_{\nu\lambda} \equiv 0 \pmod{\lambda^3}$ *then a unit in* $\Omega(\alpha)$ *which is congruent modulo* λ^2 *to a rational integer is the* λ_{th} *power of a unit in* $\Omega(\alpha)$.

In the argument covering this (p. 46), there is used the incorrect formula (2) of this paper. Also, on page 50, under assumption I, is given the

Theorem III. *If* $F(\alpha)$ *is an integer in the field* $\Omega(\alpha + \alpha^{-1})$ *and if*

$$\frac{d_o^{2\nu} lF(e^v)}{dv^{2\nu}} \equiv 0 \pmod{\lambda}$$

then a unit $E(\alpha)$ *in* $\Omega(\alpha)$ *can be found such that*

$$E(\alpha)F(\alpha) \equiv a \pmod{\lambda}$$

where a *is a rational integer.*

In proving the above, the incorrect relation (2) is again used.

In paragraphs 4, 5 and 6, pp. 50–61, Kummer goes through a course of reasoning which leads him to

Theorem IV. *If* P *is an ideal such that* P^λ *is the principal ideal* $(F(\alpha))$, *and* $B_\nu \equiv 0 \pmod{\lambda}$ *then* P *is or is not a principal ideal according as*

$$\frac{d_o^{\lambda-2\nu} lF(e^v)}{dv^{\lambda-2\nu}} \equiv \text{or } 0 \pm \pmod{\lambda}.$$

In this connection note the formula (A) which he gives on page 53. The number $\Psi_\nu(\alpha)$ which appears here is defined as the product of certain

ideal factors (11th line from bottom); but this decomposition holds for the case where $\Psi_r(\alpha)$ contains only ideals of the first degree. Kummer has himself given (*Crelle*, **44**, p. 120) the decomposition for the generalized function $\Psi_r(\alpha)$ which may contain ideals of other degrees than the first and said decomposition is of a more general form than that which applies for ideals of the first degree. It would follow from these facts that in theorem IV, $F(\alpha)$ would necessarily contain factors of the first degree only. However, on page 67 of the 1857 memoir the theorem is applied to certain ideals $\Theta_r(\alpha)$ which are not proved to be of the first degree.

Also, on page 54 it is stated without proof or reference that if m is not divisible by λ-1 then

$$\frac{d_o^{m\lambda^r} l\phi(e^v)}{dv^{m\lambda^r}} \equiv \frac{d_o^{m\lambda^r} l\phi_1(e^v)}{dv^{m\lambda^r}} \pmod{\lambda^{r+1}}$$

where $\phi(\alpha)$ and $\phi_1(\alpha)$ are integers in $\Omega(\alpha)$ such that $\phi(\alpha) \equiv \phi_1(\alpha) \pmod{\lambda^{r+1}}$. The writer has not been able to find a proof of this any where in Kummer's papers.

AN IMPROVED FORM OF HIGH TENSION D. C. APPARATUS

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In a recent paper on X-rays in these Proceedings,[1] I described a high tension D. C. outfit based on that of A. W. Hull, with certain changes of details. On further investigation it has appeared that one of these changes introduces a disturbing factor, which was not large enough to affect the results in that paper, or to be detected by the tests for fluctuation used at that time, but which might have been detectable if the choke coils in the apparatus had not had extremely high inductances. As I have received several inquiries lately about the best way to build such an outfit and the question is of importance to X-ray research workers, I am writing this note to call attention to the existence of this disturbing factor and the way to eliminate it in outfits with inductances and capacities not so large as those used here.

In the arrangement described in the previous paper the principal A. C. components on the D. C. line had a frequency of 1000 cycles per second, twice that of the primary current, and the currents in both sides of the line increased and decreased in the same phase. The disturbing factor was the capacity of the transformer secondary, relative to the ground, that introduced components of current at 500 cycles, with opposite phases on the two sides of the line. Because of this phase relation, the 500 cycle components are difficult to choke off unless the middle points of the D. C. condenser lines are grounded, in which case the phase relation of each type

of current components is unchanged, but the 500 cycle voltage fluctuations on the first D. C. condenser are increased, and its 1000 cycle voltage fluctuations are reduced. To make this change we must remove the ground connection that was on the point A, figure 1, and ground the middle points of all the D. C. condensers as indicated by the grounds, G, and incidentally, we may as well ground the cores of the choke coils also. Since the 500 cycle components have a phase relation opposite to that of the 1000 cycle ones it is well also to reverse the connections on the choke coils on one side of the line. This gives us the arrangement shown in figure 1.

To compute the potential fluctuations, let $I \sin \omega t$ be the current in the lead to either end of C_1, where $\omega = 2\pi \times 500$ sec.$^{-1}$, and we neglect harmonics. This gives potentials as indicated in the diagram, where $(V + V_1)$ is the peak voltage of the transformer,

$$V_1 = \frac{I}{2C_1\omega}, \quad V_2 = \frac{V_1}{C_2L_1\omega^2} = \frac{I}{2C_1C_2L_1\omega^3}$$

and

$$V_3 = \frac{V_2(1 - C_2M\omega^2)}{C_3L_2\omega^2} = \frac{I(1 - C_2M\omega^2)}{2C_1C_2L_1L_2\omega^5}.$$

Here C_1, C_2 and C_3 are the capacities of the whole series condenser lines, and $L_1 = 2L'_1 + 2M_1$ $L_2 = 2L'_2 + 2M_2$, and M is the mutual inductance between L_1 and L_2. Because of the ω^5 in the denominator of V_3 it is evident that harmonics need not be considered, although for the 1000 cycle harmonic we must replace L_1 and L_2 by $2L'_1 - 2M_1$ and $2L'_2 - 2M_2$, respectively. To find I for a given value of the direct current I' is not easy, and the ratio of the two is not constant. But if the rise of voltage on either half of the first condenser occurred instantaneously it would not be greater than $(2\pi/\omega)I' \div 2C_1$; and, if it took a whole half cycle it would not be less than half this amount. In practice, with heavy loads the rise appears to take a large fraction of a half cycle. The slower the rise is, the less of it we may ascribe to harmonics, and thus for an approximate value of I we may assume the rise to be $2V_1$, and also to be somewhat greater than $\pi I'/2C_1\omega$. Thus I should be about $2I'$ under such conditions, and measurements confirm this relation.

This arrangement has the disadvantage of depending on a 500 cycle charging current in C_1 instead of 1000. On the other hand, if the two sides of the line are symmetrical, there should be no 500 cycle fluctuation in the total potential difference between the ends of the line. In fact with the previous type of connection we might expect that if the ground wire was removed we should have likewise no 500 cycle fluctuation in the potential difference between the ends of the line, although both ends would fluctuate relative to the ground. But this conclusion is based on the assumption of perfect symmetry between the two ends of the line, which is liable not to occur. And although the 500 cycle fluctuation on the first

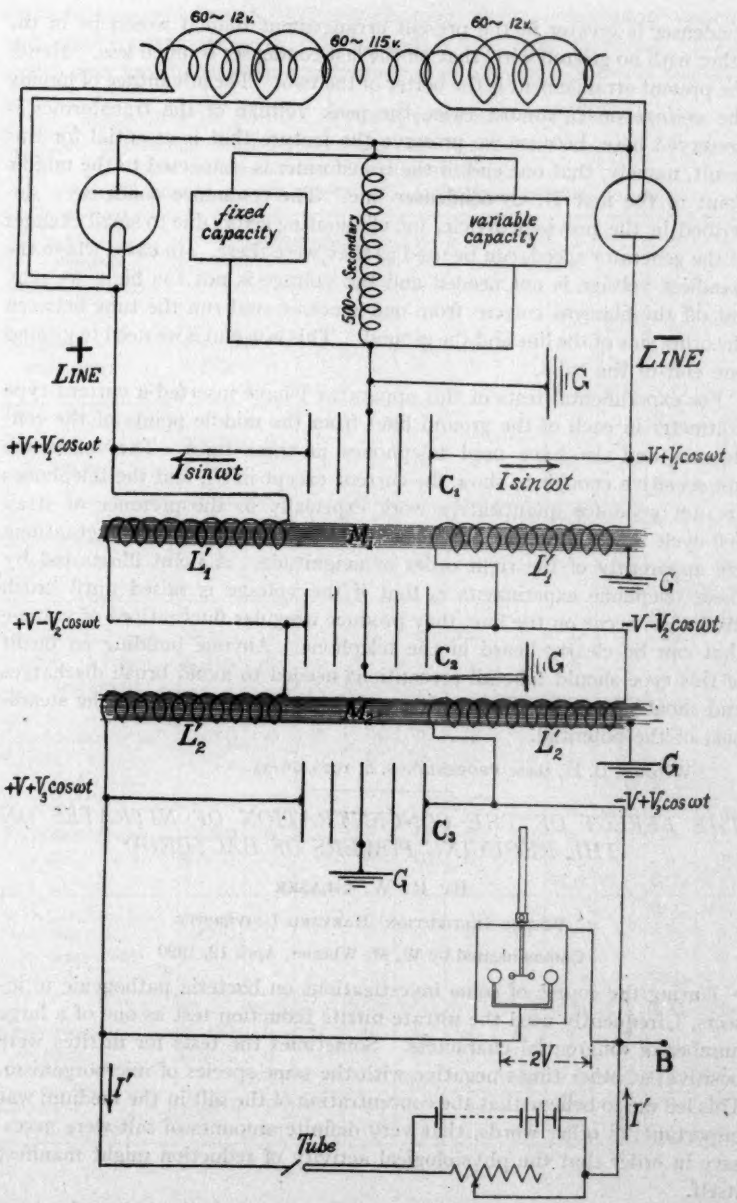
60~ 12v.
60~ 115v.
60~ 12v.
fixed capacity
500~ Secondary
variable capacity
+ LINE
− LINE
G
+V+V₁cosωt
I sinωt
C₁
I sinωt
−V+V₁cosωt
M₁
L'₁
L'₁
G
+V−V₂cosωt
C₂
G
−V−V₂cosωt
M₂
L'₂
L'₂
G
+V+V₃cosωt
C₃
G
−V+V₃cosωt
2V
B
I'
I'
Tube

FIG. 1

condenser is greater in the present arrangement than it would be in the other with no ground wire, that on the last condenser is much less. Hence the present arrangement is the better of the two. The advantage of having the voltage on C_1 almost twice the peak voltage of the transformer is preserved here, because we preserve the feature that is essential for this result, namely, that one end of the transformer is connected to the middle point of the first D. C. condenser line. The resonance condensers, described in the previous article, for eliminating errors due to small changes of the generator speed, can be used as they were there. In cases where the steadiest voltage is not needed and the voltage is not too high, we may cut off the filament current from one kenotron and run the tube between the other side of the line and the ground. This is useful if we need to ground one end of the tube.

For experimental tests of this apparatus I have inserted a current-type voltmeter in each of the ground lines from the middle points of the condensers, and also have used telephones in these lines. The voltmeters not sensitive enough to show the current except in C_1, and the telephones are not good for quantitative work expecially in the presence of stray 500 cycle noises, but the currents and, therefore, the voltage fluctuations are apparently of the right order of magnitude. A point illustrated by these telephone experiments is that if the voltage is raised until brush discharges occur on the line, they produce irregular fluctuations of voltage that can be clearly heard in the telephone. Anyone building an outfit of this type should take all precautions needed to avoid brush discharges and should listen for them with a telephone before relying on the steadiness of the potential.

[1] Webster, D. L., these PROCEEDINGS, 6, 1920, 26–35.

THE EFFECT OF THE CONCENTRATION OF NITRATES ON THE REDUCING POWERS OF BACTERIA[1]

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During the course of some investigations on bacteria pathogenic to insects, I frequently used the nitrate-nitrite reduction test as one of a large number of differential characters. Sometimes the tests for nitrites were positive; at other times negative with the same species of microörganism. This led me to believe that the concentration of the salt in the medium was important; in other words, that very definite amounts of salt were necessary in order that the physiological activity of reduction might manifest itself.

As shown in the accompanying table a number of species of micro-

organisms were selected. Witte's peptone media, containing various molecular concentrations of $NaNO_3$ and KNO_3, were prepared. These media were inoculated with equal amounts of 48-hour cultures of the bac-

TABLE SHOWING RESULTS OF THE NITRATE-NITRITE REDUCTION TESTS

Code: A plus sign indicates reduction of nitrates to nitrites
A cipher indicates the lack of such reduction
WG indicates a weak growth. NG indicates no growth

SPECIES OF ORGANISM		.0002 M	.0005 M	.0008 M	.001 M	.01 M	.1 M	.5 M	1 M	2 M	4 M
								W.G.	W.G.	N.G.	N.G.
Spirillum metchnikovi	$NaNO_3$	0	+	+	+	+	+	0	0	0	0
	KNO_3	0	+	+	+	+	+	0	0	0	0
								W.G.	W.G.	N.G.	N.G.
										N.G.	N.G.
Bacillus prodigiosus	$NaNO_3$	0	0	+	+	+	+	+	+	0	0
	KNO_3	0	0	+	+	+	+	+	+	0	0
										N.G.	N.G.
								W.G.	W.G.	W.G.	N.G.
Bacillus coli communis	$NaNO_3$	0	0	0	0	+	+	+	0	0	0
	KNO_3	0	0	0	0	+	+	+	0	0	0
									W.G.	W.G.	N.G.
										W.G.	N.G.
Coccobacillus acridiorum. Variety "Souche Sidit"	$NaNO_3$	0	0	0	0	+	+	+	+	0	0
	KNO_3	0	0	0	0	+	+	+	+	0	0
										W.G.	N.G.
									W.G.	W.G.	W.G.
Coccobacillus acridiorum. Variety "Souche Cham"	$NaNO_3$	0	0	0	0	+	+	+	0	0	0
	KNO_3	0	0	0	0	+	+	+	0	0	0
									W.G.	W.G.	N.G.
									W.G.	W.G.	W.G.
Bacillus anthracis	$NaNO_3$	0	0	0	0	0	0	0	0	0	0
	KNO_3	0	0	0	0	0	0	0	0	0	0
								W.G.	W.G.	W.G.	N.G.
										W.G.	N.G.
Staphylococcus pyogenes albus	$NaNO_3$	0	0	0	0	0	0	0	0	0	0
	KNO_3	0	0	0	0	0	0	0	0	0	0
										W.G.	N.G.
		W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	N.G.	N.G.	N.G.
Streptococcus disparis	$NaNO_3$	0	0	0	0	0	0	0	0	0	0
	KNO_3	0	0	0	0	0	0	0	0	0	0
		W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	W.G.	N.G.	N.G.
Checks (No bacteria)	$NaNO_3$	0	0	0	0	0	0	0	0	0	0
	KNO_3	0	0	0	0	0	0	0	0	0	0

teria concerned and incubated for five days. After this time the cultures were tested for the presence of nitrites by the use of the sulphuric acid, potassium iodide, starch reaction and the naphthylamine, sulphanilic acid, acetic acid test. Of course, the absence of nitrites may not indicate non-reduction of nitrates, since the nitrites formed prior to the test may have been reduced to free nitrogen or ammonia. Nevertheless, in that case a nitrate reaction should not be obtained. The presence or absence of nitrates after five days growth was tested in each case by evaporating the culture to dryness and by adding phenolsulphonic acid, diluting with water and transferring to a Nessler jar. Sodium hydroxide solution was added to make it alkaline and the color reactions compared.

The table shows that each species capable of reduction has a distinct range. *Bacillus prodigiosus* has a greater range than *Spirillum Metchnikovi*, but the latter has the power of reducing nitrates in a weaker concentration. *Prodigiosus*, on the other hand, has the power of reduction in greater concentrations of the salts. This inability of *Metchnikovi* to reduce in greater concentrations may be associated with the inhibiting influence of high osmotic pressure on growth, for it will be seen that *Metchnikovi* grew weakly in concentrations greater than 0.1 mol. That growth and ability to reduce are not always associated, however, can be seen in the case of *B. coli communis* which grew poorly in 0.5 mol. concentration and reduced $NaNO_3$.

A distinct range of reduction power in various concentrations can also be noted for some of the other species. Some have a smaller, some a greater range. In so far as the reducing power is concerned no difference was noted between sodium and potassium ions. Nevertheless, a slight difference between sodium and potassium ions in the amount of growth was observed. In 4 mol. $NaNO_3$ *Coccobacillus acridiorum* variety "Souche Cham" grew poorly, but no growth whatever was obtained in the same concentration of KNO_3. The same condition was observed when the Anthrax bacillus was used. This difference in ions was further observed by using *B. coli communis*, where the sodium salt gave a poor growth at 0.5 mol. concentration and the potassium salt a good growth at the same concentration. The difference between ions and growth is not dependent upon variations in the number of bacteria originally introduced into the cultures for the amounts were previously carefully calibrated.

[1] Contribution from the U. S. Bureau of Entomology in coöperation with the Bussey Institution of Harvard University. (Bussey Institution, No. 173.)